

**What is claimed is:**

1. A process comprising
  - a) providing, in a suitable container, cells that express a hyperpolarization-activated cation channel;
  - b) hyperpolarizing the cells in the presence of a potential-sensitive fluorescent dye and an isoosmolar sodium-ion-free buffer;
  - c) optionally, determining the membrane potential of the cells;
  - d) simultaneously adding sodium ions and a sample containing at least one substance to be tested for its ability to modulate the activity of the cation channel;
  - e) determining the membrane potential of the cells;
  - f) determining whether the membrane potential changed upon simultaneous addition of sodium ions and the substance(s); and
  - g) optionally, recording the change in membrane potential, wherein a change in membrane potential indicates the presence of at least one substance in the sample that modulates the activity of the cation channel.
2. The process of claim 1, wherein step c) is performed.
3. The process as claimed in claim 1, wherein the isoosmolar sodium-ion-free buffer comprises a potassium salt.
4. The process as claimed in claim 1, wherein the isoosmolar sodium-ion-free buffer comprises potassium ions at a concentration of at least 0.8 mM.
5. The process as claimed in claim 1, wherein the isoosmolar sodium-ion-free buffer comprises potassium ions at a concentration of at least 5 mM.

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6. The process as claimed in claim 1, wherein the isoosmolar sodium-ion-free buffer comprises choline chloride or NMDG (N-methyl-D-glucamine).
7. The process as claimed in claim 1, wherein the isoosmolar sodium-ion-free buffer comprises a potential-sensitive dye.
8. The process as claimed in claim 7, wherein the potential-sensitive dye is a fluorescent dye.
9. The process as claimed in claim 8, wherein the potential-sensitive dye is an oxonol derivative.
10. The process as claimed in claim 9, wherein the oxonol derivative is a 3-bis-barbituric acid oxonol.
11. The process as claimed in claim 10, wherein the 3-bis-barbituric acid oxonol is bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBac<sub>4</sub>(3)], bis-(1,3-diethylthiobarbituric acid)trimethine oxonol [DiSBac<sub>2</sub>(3)], bis-(1,3-dibutylbarbituric acid)pentamethine oxonol [DiBac<sub>4</sub>(5)], or a combination of these.
12. The process as claimed in claim 8, wherein the potential-sensitive fluorescent dye used is suitable for use in fluorescent imaging plate reader system.
13. The process as claimed in claim 1, wherein cells having an elevated intracellular cAMP concentration are used.
14. The process as claimed in claim 13, wherein the intracellular cAMP concentration is increased by addition of dibutyryl-cAMP or 8-bromo-cAMP.

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15. The process as claimed in claim 13, wherein the intracellular cAMP concentration is increased by addition of an adenylate cyclase activator.
16. The process as claimed in claim 13, wherein the intracellular cAMP concentration is increased by addition of forskolin.
17. The process as claimed in claim 16, wherein the intracellular cAMP concentration is increased by addition of from 1  $\mu$ M to 100  $\mu$ M of forskolin.
18. The process as claimed in claim 13, wherein the intracellular cAMP concentration is increased by addition of receptor ligands.
19. The process as claimed in claim 1, wherein the hyperpolarization-activated cation channel is HCN1, HCN2, HCN3, HCN4, KAT1, or a heteromultimer of these channels.
20. The process as claimed in claim 1, wherein the hyperpolarization-activated cation channel is a human hyperpolarization-activated cation channel.
21. The process as claimed in claim 1, wherein the cells are mammalian cells.
22. The process as claimed in claim 21, wherein the cells are CHO or HEK cells.
23. The process as claimed in claim 1, wherein the cells contain a plasmid which comprises the cDNA of a hyperpolarization-activated cation channel.
24. The process as claimed in claim 23, wherein the cells comprise a second plasmid, which comprises the cDNA of the same hyperpolarization-activated cation channel.

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25. The process as claimed in claim 23, wherein the cells comprise a second plasmid, which comprises the cDNA of a different hyperpolarization-activated cation channel, such that heteromultimeric HCN channels can be formed.
26. The process as claimed in claim 1, wherein the cells comprise a plasmid, which comprises synthetic cDNA encoding at least part of at least two different cation channels.
27. The process as claimed in claim 1, wherein a change in membrane potential is measured using a potential-sensitive fluorescent dye.
28. The process as claimed in claim 27, wherein the potential-sensitive fluorescent dye is an oxonol derivative.
29. The process as claimed in claim 28, wherein the oxonol derivative is 3-bis-barbituric acid oxonol.
30. The process as claimed in claim 1, wherein at least one measurement is carried out in a Fluorescent Imaging Plate Reader (FLIPR).
31. The process as claimed in claim 1, wherein the change of the membrane potential of at least two cells is compared.
32. The process as claimed in claim 1, wherein the process is a high-throughput screening process.

33. A process, said process comprising
- a) providing, in a suitable container, cells that express a hyperpolarization-activated cation channel;
  - b) hyperpolarizing the cells in the presence of a potential-sensitive fluorescent dye and an isoosmolar sodium-ion-free buffer;
  - c) optionally, determining the membrane potential of the cells;
  - d) incubating the cells with a sample containing at least one substance to be tested for its ability to modulate the activity of the cation channel;
  - e) optionally, determining the membrane potential of the cells;
  - f) optionally, determining whether the membrane potential changed upon addition of the substance(s) to be tested;
  - g) adding sodium ions;
  - h) determining the membrane potential of the cells;
  - i) determining whether the membrane potential changed upon addition of the sodium ions; and
  - j) optionally, recording the change in membrane potential,
- wherein a change in membrane potential between the time before the sodium ions are added and after the sodium ions are added indicates the presence of at least one substance in the sample that modulates the activity of the cation channel.
34. The process as claimed in claim 33, wherein steps c) and e) are performed.
35. The process as claimed in claim 33, wherein the isoosmolar sodium-ion-free buffer comprises a potassium salt.
36. The process as claimed in claim 33, wherein the isoosmolar sodium-ion-free buffer comprises potassium ions at a concentration of at least 0.8 mM.

37. The process as claimed in claim 33, wherein the isoosmolar sodium-ion-free buffer comprises potassium ions at a concentration of at least 5 mM.
38. The process as claimed in claim 33, wherein the isoosmolar sodium-ion-free buffer comprises choline chloride or NMDG (N-methyl-D-glucamine).
39. The process as claimed in claim 33, wherein the isoosmolar sodium-ion-free buffer comprises a potential-sensitive dye.
40. The process as claimed in claim 39, wherein the potential-sensitive dye is a fluorescent dye.
41. The process as claimed in claim 40, wherein the potential-sensitive dye is an oxonol derivative.
42. The process as claimed in claim 41, wherein the oxonol derivative is a 3-bis-barbituric acid oxonol.
43. The process as claimed in claim 42, wherein the 3-bis-barbituric acid oxonol is bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBac<sub>4</sub>(3)], bis-(1,3-diethylthiobarbituric acid)trimethine oxonol [DiSBac<sub>2</sub>(3)], bis-(1,3-dibutylbarbituric acid)pentamethine oxonol [DiBac<sub>4</sub>(5)], or a combination of these.
44. The process as claimed in claim 40, wherein the potential-sensitive fluorescent dye used is suitable for use in fluorescent imaging plate reader system.
45. The process as claimed in claim 33, wherein cells having an elevated intracellular cAMP concentration are used.

46. The process as claimed in claim 45, wherein the intracellular cAMP concentration is increased by addition of dibutyryl-cAMP or 8-bromo-cAMP.
47. The process as claimed in claim 45, wherein the intracellular cAMP concentration is increased by addition of an adenylate cyclase activator.
48. The process as claimed in claim 45, wherein the intracellular cAMP concentration is increased by addition of forskolin.
49. The process as claimed in claim 48, wherein the intracellular cAMP concentration is increased by addition of from 1  $\mu$ M to 100  $\mu$ M of forskolin.
50. The process as claimed in claim 45, wherein the intracellular cAMP concentration is increased by addition of receptor ligands.
51. The process as claimed in claim 3, wherein the hyperpolarization-activated cation channel is HCN1, HCN2, HCN3, HCN4, KAT1, or a heteromultimer of these channels.
52. The process as claimed in claim 33 wherein the hyperpolarization-activated cation channel is a human hyperpolarization-activated cation channel.
53. The process as claimed in claim 33, wherein the cells are mammalian cells.
54. The process as claimed in claim 33, wherein the cells are CHO or HEK cells.
55. The process as claimed in claim 33, wherein the cells contain a plasmid which comprises the cDNA of a hyperpolarization-activated cation channel.

56. The process as claimed in claim 55, wherein the cells comprise a second plasmid, which comprises the cDNA of the same hyperpolarization-activated cation channel.
57. The process as claimed in claim 55, wherein the cells comprise a second plasmid, which comprises the cDNA of a different hyperpolarization-activated cation channel, such that heteromultimeric HCN channels can be formed.
58. The process as claimed in claim 33, wherein the cells comprise a plasmid, which comprises synthetic cDNA encoding at least part of at least two different cation channels.
59. The process as claimed in claim 33, wherein a change in membrane potential is measured using a potential-sensitive fluorescent dye.
60. The process as claimed in claim 59, wherein the potential-sensitive fluorescent dye is an oxonol derivative.
61. The process as claimed in claim 60, wherein the oxonol derivative is 3-bis-barbituric acid oxonol.
62. The process as claimed in claim 33, wherein at least one measurement is carried out in a Fluorescent Imaging Plate Reader (FLIPR).
63. The process as claimed in claim 33, wherein the change of the membrane potential of at least two cells is compared.
64. The process as claimed in claim 33, wherein the process is a high-throughput screening process.



65. A kit, said kit comprising
- a) cells that overexpress a hyperpolarization-activated cation channel;
  - b) an isoosmolar sodium-ion-free buffer for hyperpolarizing the cell; and
  - c) at least one reagent for detection of hyperpolarization activated cation channels.

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